Influence of sirolimus-induced TGF-β secretion on mouse Treg cell proliferation

J.N. Li[1]*, J.X. Li[1]*, H.L. Huang[1]*, X.L. Zhu[1], H. Ding[1], C.F. Huang[1], J. Lin[1], J.G. Huang[1], Z.C. Wu[1], M. Ashraf[2], Y.G. Wang[3], X.K. Li[4], S.Y. Zheng[1], J.M. Chen[1], H.M. Guo[1], J. Zhuang[1] and P. Zhu[1]

[1]Department of Cardiovascular Surgery, Guangdong Cardiovascular Institute, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, China
[2]Department of Pharmacology, UIC College of Medicine, Chicago, IL, USA
[3]Department of Pathology & Lab Medicine, College of Medicine, University of Cincinnati, Cincinnati, OH, USA
[4]Division of Transplantation Immunology, National Research Institute for Child Health and Development, Tokyo, Japan

*These authors contributed equally to this study.
Corresponding authors: J. Zhuang / P. Zhu
E-mail: zhuangjianzggd@aliyun.com / tanganqier@163.com

Genet. Mol. Res. 14 (4): 18569-18579 (2015)
Received January 8, 2015
Accepted July 10, 2015
Published December 28, 2015
DOI http://dx.doi.org/10.4238/2015.December.28.4

ABSTRACT. We examined the effects of co-culturing CD4+ CD25+ Treg cells with sirolimus or cyclosporin A on Treg cell proliferation and differentiation and on transforming growth factor-β (TGF-β) and Foxp3 expression. CD4+ CD25+ Treg cells were harvested from mononuclear cells of spleens of C57BL/6 mice using immunomagnetic beads and divided into control, sirolimus, and cyclosporine groups. Following a 96-h co-culture, Treg cells were assayed by flow cytometry. FoxP3 and TGF-β mRNA levels and secretion were assayed by reverse transcription polymerase chain reaction and enzyme-linked immunosorbent assay. Smad protein of the TGF-β signaling pathway was assayed by western blot and its effect on CD4+ CD25+ FoxP3+ Treg cell proliferation was

determined. Sirolimus-promoted differentiation and proliferation was examined using a TGF-β neutralizing antibody. Sirolimus-treated CD4+ T cell TGF-β secretion increased 2.5X over control levels ($P < 0.01$), but that of the cyclosporine group decreased marginally ($P > 0.05$). The CD4+ cell proportion decreased significantly (41.25 *vs* 69.22%, $P < 0.01$) and slightly (65.21 *vs* 69.22, $P > 0.05$) in the cyclosporine and sirolimus groups, respectively. T cell *Foxp3* mRNA expression was significantly higher in the sirolimus-treated than in the cyclosporine (53.7 *vs* 40.2%, $P < 0.05$) and control groups ($P < 0.01$), but was significantly lower in the cyclosporine group than in controls (23.6 *vs* 40.2%, $P < 0.01$). Overall, sirolimus promoted CD4+ CD25+ Treg cell proliferation and growth *in vitro*, whereas cyclosporin A inhibited proliferation. Sirolimus might promote CD4+ CD25+ FoxP3+ regulatory T cell proliferation by inducing TGF-β secretion *in vivo.*

Key words: Sirolimus; Cyclosporin A; Regulatory T cells; Foxp3; Mice; Transforming growth factor-β

